THE INTERRELATION OF FERRIC IONS AND FLUORIDE IONS IN ANIMAL NUTRITION

by

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B. S., Washburn University, 1932

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1954

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INTRODUCTION

The lack of information on the influence of the fluoride ion in iron metabolism prompted this investigation. A search of the literature disclosed very little pertinent data on this particular problem even though there is a wealth of published information concerning the effect of fluorine and its compounds (Smith et al., 19) particularly in relation to dentition, and upon other phases of iron metabolism (Moore, 15). The influence of fluorine compounds upon the metabolism of other mineral elements is of particular importance in animal nutrition because of the increasing utilization of commercial feeds which often contain ingredients of relatively high fluorine content. This study was an exploratory investigation designed to delineate the influence of the fluoride ion upon the absorption and utilization of iron by determining the influence of the fluoride ion upon radioactive iron previously administered directly by stomach tube, and by noting the effect of the fluoride ion when administered as a supplementary component of the basic diet.

Hegsted et al. have investigated the influence of diet on iron absorption in a series of studies (10) (11) (12). Their data substantiate the conclusion of Hahn et al. (7) that iron absorption is normally controlled by a mechanism which regulates the absorption of iron in proportion to body needs, and indicates further that there is normally a block to iron absorption which might be overcome by certain types of diet. They have shown (11) that iron absorption can be changed by altering either the phosphorus or iron content of the diet. Their data suggests also that

some materials in the diet, for instance certain amino acids, might increase the amount of iron absorbed.

Granick (5) postulated a mechanism of iron absorption, transport and function as follows: Iron (III) enters the gastrointestinal tract with the food and is converted to the iron (II) by reducing agents in the food and in the tissues. Absorption of iron (II) then occurs, mainly in the mucosal cells of the duodenum and jejenum. The presence of iron evokes an increase in concentration of apo-ferritin which combines with the iron to form ferritin which in turn accumulates as reservoir iron. Ferritin, an iron containing protein, may be the receptor compound postulated by Hahn et al. (7) which, in the intestinal mucosa, is capable of reversibly combining with iron by taking up limited amounts from the intestinal lumen and then passing it on to the plasma.

Sharpe et al. (18) added ascorbic acid to the diet to reduce iron (III) to iron (II). This work, designed primarily to explore the effects of phytate and other food factors upon iron absorption, showed that sodium phytate reduced iron absorption by 93 percent and indicated an inverse correlation between iron absorption and the solids content of test meals fed boys 12 to 17 years of age. Nissim (16) reported that the iron distribution in the rat and mouse, after injections of "saccharated iron oxide", corresponded very closely to that of ascorbic acid in the adrenals, ovaries and young connective tissue. Ruskin and Merrell (17) studied the reactions of iron and ascorbic acid and suggested, on the basis of their studies that the biological value of iron preparations is important and that the accumulation of large amounts of iron of low biological value is

dangerous and may cause iron cirrhosis. Their work indicated that an iron (II)-iron (III) complex forms with ascorbic acid similar to that proposed for oxalic acid.

Gullberg and Vahlquist (6) reported that molybdenum did not improve the absorption of iron. Tomaselli (20) reported that serum iron and the iron content of liver, bone marrow and lungs increased in lead poisoned animals. Numerous studies have been reported upon the influence of copper in iron metabolism. Chase et al. (1) reported that there was less absorption of iron from the gastrointestinal tract of rats deficient in copper than in rats supplied with copper. Ventkataramanan and Krishnaswamy (22) showed that the addition of aluminum sulfate to the diet of rats receiving fluorine compounds markedly inhibited the onset of severe dental changes.

In studies of systems containing iron and fluoride ions, Dodgen and Rollefson (2) demonstrated in acid solution that increasing amounts of first FeF^{++}, then FeF_2^+ and FeF_3 are formed as very small fluoride ion concentrations are increased. Hudis and Wahl (13) studied the kinetics of the exchange reactions in vitro between the iron (II) ion and fluoride complexes of iron (III). The values of the formation constants of iron(III) fluoride complexes are large, and the complexes can be formed at very low concentrations of fluoride ion. If iron must be in the iron (II) state for absorption it is possible that exchange and the formation of fluoride complexes would interfere with the absorption of iron when fluoride ion is present in the diet.

Ginn and Volker (4) reported that one of the effects of the fluoride ion ingestion by the rat was a pronounced reduction of blood hemoglobin,

whereas McClure and Kornberg (14) found that the fluoride ion had no effect on hemoglobin and hematocrit values of rat blood.

This study was undertaken because of the divergent conclusions of these reports, and the possible formation of iron-fluoride complexes which might subsequently interfere with iron metabolism.

EXPERIMENTAL

Feeding and Management

Weanling male rats of the Sprague-Dawley strain were used in this study. As the shipment arrived much earlier than anticipated it was necessary to maintain these animals in group cages on stock diet (Purina Rabbit Chow) until the special cage facilities were completed.

These animals were transferred to the special cages and placed on the experimental diets at a weight range of 100 to 130 grams.

It was necessary to hold the animals on the experimental diets for a total of 77 days, much longer than planned, due to delays in shipment of the radioactive iron. During this period the rats attained weights ranging from 150 to 275 grams. Although it was originally intended to administer the radioactive iron during a period of rapid growth, these plans were abandoned because of the initial early receipt of older animals and the subsequent delays encountered. In view of the blood pictures which developed, these delays were fortituous in that they allowed sufficient time to develop a state of iron deficiency which might otherwise not have been reached.

Cage Construction and Modifications

To avoid ingestion of iron from extraneous sources all animals were placed on glass, in plastic cages. The glass cage bottoms, illustrated in Plate I, were assembled from wood and glass tubing. The wood support rails were drilled so that the two outer glass tubes were a press-fit, whereas the balance of the tubes were loose-fit, thus providing a self-supporting assembly and precluding the necessity for external tie-rods. Plastic mouse cages were inverted and used to complete the cage structure, as shown in Plate II. Retaining clips, shown in Plate I, were necessary to prevent the animals from pushing these cages off the glass rack.

Excessive condensation occurred in these cages when more than two rats were housed in a single cage and it was necessary to drill a series of ventilation holes along the sides and in the tops of the plastic covers. Even with these precautions it was necessary to change the litter daily, or at the very least on alternate days, to avoid excessive condensation and wet animals.

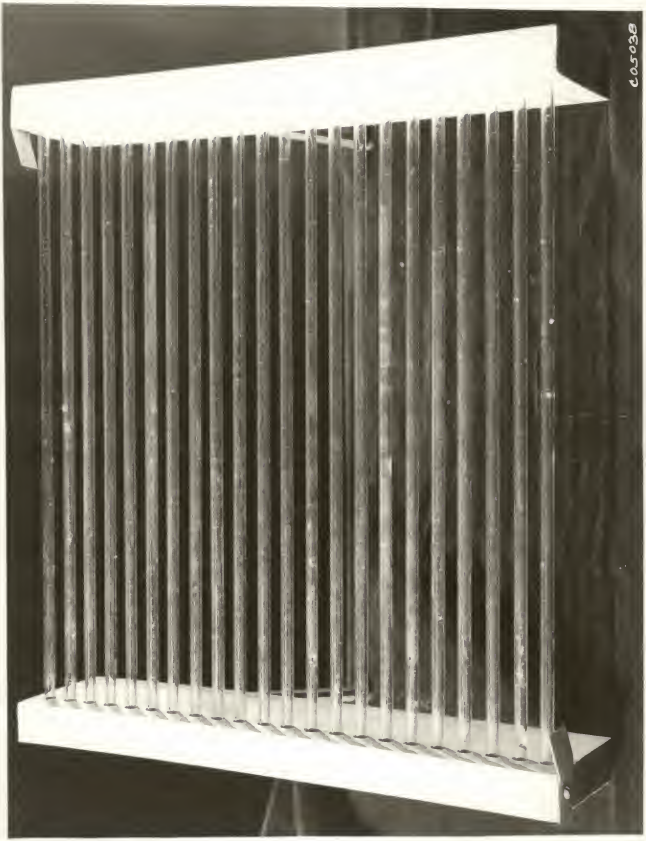
Glazed ceramic feeding crocks of the type shown inside the cage in Plate II proved most satisfactory for this application where feed consumption was not measured. Excessive feed loss did occur. In any application where feed intake was a factor and loss had to be measured a non-scatter type jar would be essential.

Water was supplied through the glass storage bottle and drip type assembly shown suspended on the racks in Plate III. The tip was inserted through a hole in the plastic cover which served for both positioning and support.

EXPLANATION OF PLATE I

Photograph of the cage support rack.

PLATE I



EXPLANATION OF PLATE II

Photograph of the cage assembly.

PLATE II

EXPLANATION OF PLATE III

Photograph showing the cage rack and the experimental assembly.

PLATE III

The special cages described above were constructed to fit the pans of a standard cage rack assembly as shown in Plate III. Because of limited space, equipment, and facilities, animals were housed in groups of four for this exploratory study. This arrangement prevented collection of excreta from individual animals during the period of radioactive iron administration, but this was not otherwise undesirable. More precise studies of certain specific phases of this problem would necessitate individual cages.

Basal Diet

Harris (8) reported a method for the production of nutritional anemia in the rat. The diet which he describes formed the basis of the diet employed in these studies. The diet was designed to achieve a reasonable degree of iron depletion in the rat without incurring excessive mortality. This diet is hereafter referred to as the basal diet.

Eight groups of four animals each and a group of five spare animals were maintained on the basal experimental diet which consisted of dried skimmed milk supplemented with 10 ppm of copper, added as $CuSO_4.5H_2O$ in 10 ml of water, two percent corn oil to which Vitamin A oil was added to provide 5 IU of Vitamin A per gram of diet, and Vitamin D oil to provide 3 USP units of Vitamin D_2 per gram of diet. These supplementary components were mixed into the milk base by a series of successive dry dilutions, initially by mulling in a morter and, as dilution proceeded, by rolling and quartering. Diets were prepared in one kilogram batches.

Supplemented Diets

Two groups of four animals each were maintained on the basal diet supplemented with 50 ppm iron, added as ferric chloride by dissolving in 10 ml water and then mixed into the diet by the techniques described previously.

Two groups of four animals each received, in addition to the 50 ppm of iron, 200 ppm fluoride ion added as sodium fluoride. The ferric chloride and sodium fluoride required 20 ml water as a dispersing agent and even then a portion of the sodium fluoride was merely dispersed rather than dissolved. It was impractical to use enough water to completely dissolve the sodium fluoride as such an addition exceeded permissible moisture in the dried milk product. These two groups received an approximate ratio of 12 moles fluoride ion to one mole of iron.

Diet was fed ad libitum. Under most conditions four jars of feed per cage (approximately 60 grams) provided more than sufficient diet for a 24 hour period, but feed jars were checked at more frequent intervals and filled as necessary. Uncontrollable losses under the special conditions imposed account for this high feed requirement.

Administration of Solutions

All radioactive material was administered by stomach tube in doses of 0.5 ml per feeding. This amount of fluid was measured in and expressed by means of a Luer-lock syringe. After each dose of radioactive material

was administered, and prior to removal of the stomach tube, a syringe containing 0.5 ml of distilled water was attached. This volume of water was injected to rinse residual radioactive solution into the stomach.

Polyethylene tubing was used for the stomach tube. It was fully satisfactory with the exception that the animal had to be carefully held with complete jaw restraint, otherwise the tubing was easily perforated by the rat's incisors. Difficulty was experienced in some instances in inserting the stomach tube in rats on the fluoride diet, since the typical incisor growth of rats on a diet containing sodium fluoride (19) obstructed the normal tube insertion path. An 18 gauge needle, one inch in length, was used as a connector between the syringe and the stomach tube. The needle and tube remained assembled while syringes were changed without removal of the stomach tube. In this way the different solutions could be administered without delay and without the difficulty of tube reinsertion.

Radioactive Iron Feeding

Beginning on the 77th day of maintenance on experimental diets, radioactive iron was administered, both with and without added fluoride ion. For this study, 2 millicuries of Fe^{59}, as ferric chloride, in 0.32N hydrochloric acid solution was procured from Oak Ridge. The material furnished had a specific activity of 3667 millicuries per gram at time of shipment (3 P.M., July 23, 1953), and 1.1 ± 10 percent millicuries per ml. The solution contained less than 2.2×10^{-3} millicurie per ml of Fe^{55} and less than 1×10^{-4} millicurie of Co^{60} per ml.

Solutions of Fe^{59} were adjusted for administration in 0.5 ml doses. Solutions for feeding one time only contained 34 microcurie Fe^{59} activity per dose, solutions for feeding twice contained 17 microcurie Fe^{59} activity, and solutions for feeding three times contained 11.3 microcurie of Fe^{59} activity. Each solution was adjusted with non-radioactive iron to contain a total of 100 micrograms iron per 0.5 ml dose to provide adequate iron for maximum absorption in iron deficient rats. In those groups where fluoride ion was also administered, 0.5 ml of a separate fluoride ion solution was given immediately after the iron solution without changing the stomach tube, in place of the rinsing dose of 0.5 ml water mentioned above. Fluoride ion was added, as sodium fluoride, in stoichiometric amounts calculated to form the complex $FeF_6^{\blacksquare}$, which is probably the iron-fluoride complex that requires the greatest amount offluoride ion.

Animals were administered the radioactive material and fluoride ion solution and sacrificed for tissue recovery in accordance with the following schedule:

Group I, four animals receiving basal diet only, was administered 34 microcuries Fe^{59} and sacrificed at 8 hours.

Group II, four animals receiving basal diet only, was administered 34 microcuries Fe^{59} plus fluoride solution, and sacrificed at 8 hours.

Group III, four animals receiving basal diet only, was administered 34 microcuries Fe^{59} and sacrificed at 24 hours.

Group IV, four animals receiving basal diet only, was administered 34 microcuries Fe^{59} plus fluoride solution and sacrificed at 24 hours.

Group V, four animals receiving basal diet plus 50 ppm iron, was administered 34 microcuries Fe^{59} and sacrificed at 24 hours.

Group VI, four animals receiving basal diet plus 50 ppm iron and 200 ppm fluoride, was administered 34 microcuries Fe^{59} and sacrificed at 24 hours.

Group VII, four animals receiving basal diet only, was administered an initial feeding of 17 microcuries of Fe^{59}, a second feeding of 17 microcuries of Fe^{59} 24 hours later, and sacrificed at 48 hours.

Group VIII, four animals receiving basal diet only, was administered an initial feeding of 17 microcuries Fe^{59} plus fluoride solution, a second feeding of 17 microcuries of Fe^{59} plus fluoride solution 24 hours later, and sacrificed at 48 hours.

Group IX, four animals receiving basal diet only, was given an initial feeding of 11.3 microcuries Fe^{59}, a second feeding of 11.3 microcuries at 24 hours, a third feeding of 11.3 microcuries at 48 hours and was sacrificed at 72 hours.

Group X, four animals receiving basal diet only, was given an initial feeding of 11.3 microcuries Fe^{59} plus fluoride solution, an identical feeding at 24 hours, a third feeding at 48 hours, and sacrificed at 72 hours.

Group XI, four animals receiving the basal diet plus 50 ppm iron, was given an initial feeding of 11.3 microcuries of Fe^{59}, an identical feeding at 24 hours, and a third such feeding at 48 hours, then sacrificed at 72 hours.

Group XII, four animals receiving the basal diet plus 50 ppm iron plus 200 ppm fluoride, was administered an 11.3 microcurie feeding of Fe^{59} initially, and identical feedings at 24 and 48 hours, then sacrificed at 72 hours.

Rat #13 died less than two hours following Fe^{59} administration due to reasons unknown. Rat # 21 was killed during administration. Upon opening the abdominal cavity it was noted that the gastrointestinal tract was severely distended.

Because of some of the difficulties encountered in administration of fluids by stomach tube, rats #24 and #50 received only a part of the single dose alloted, while rats #51 and #53 did not receive a full second dose, and rat #3 received no third dose. Rat #25 received only 80 percent of the dose scheduled, rat #39 received only 28 percent of the second dose, and rat #48 received only 86 percent of the third dose because of shortage of radioactive iron solution. The tissue values given in Table A1, were corrected for these reduced doses.

Analytical Procedures

Biological. Animals were sacrificed at periods of 8, 24, 48, and 72 hours after the initial feeding of Fe^{59}, in accordance with the schedule outlined above. All animals were immobilized by deep ether anesthesia. The viscera were exposed by executing a longitudinal incision from the abdominal area cephalically to the tracheal area, and caudally to the perineum. Lateral incisions through the sidewalls just caudal to the rib cage facilitated removal of tissue specimens. The liver, gut, and spleen were

removed, weighed, and immersed in nitric acid. Gut contents were separated from the gut by scraping and washing. Due to difficulties involved in recovering these contents, it was not possible in this study to control wash water with sufficient accuracy to obtain weights of the gut content. Since tissues were not perfused, all tissue measurements include residual blood. A small sample of blood was obtained from each animal during tissue removal. This was also weighed in order to determine the specific activity at a later time. The balance of the blood, and all other carcass components, were pooled and treated as residual carcass.

All separated tissues and each carcass were individually digested in nitric acid until a clear solution was obtained, then reduced in volume by evaporation to drive off the bulk of the nitric acid.

Blood for hemoglobin and hematocrit determinations was obtained by tail puncture except at time of sacrifice, when blood was obtained from the body cavity.

Radioactive. All samples were counted in the liquid form by a Model TGC5 Tracerlab Geiger Mueller tube in a 25 ml dipping counter reservoir, using a Berkeley Model 2000 Scaler. The tube had a nominal window thickness of 30 mg/cm^2, was operated at 875 volts, and had a rated recovery time of 90 microseconds. Great care was taken to hold constant the counting geometry of the assembly. Reference samples were used periodically and tissue counts adjusted to a standard basis by using the ratio of the reference count to calculated standard count compensated for decay of the Fe^{59}. In addition all values were corrected for background count.

Background counts ranged from 42 to 60 cpm. Actual counting rates for the bulk of the samples were under 25,000 cpm. In a very few cases

this was exceeded with counts ranging to 75,000 cpm. Most of the tissues were counted at rates below 10,000 cpm. Computed dead time corrections for most samples were of the order of two to five percent, with a maximum of 12 percent for the high counts. Dead time adjustments were not made in the data shown herein.

Due to the collapse of the shield structure, and destruction of the counter tube just prior to counting the last sample, carcass sample #9 was counted on a new tube. Comparative counts on reference solutions of Fe^{59} were used to correct the values for this sample to the same base as counts on the other tube.

After the digest from individual tissues was evaporated, it was cooled and diluted with water to 25 ml for measurement. In some cases it was necessary to dilute to 50 ml. In such instances a 25 ml aliquot was measured and the resultant counts multiplied by two. Small amounts of ether or alcohol were necessary in a few cases to dissolve refractory components of the digest. The amount of these agents utilized was small enough to be negligible in their effect upon counting geometry. The digest from the carcass contained large amounts of fats and some mineral components which precluded reduction of the entire volume of solution to 25 ml. Consequently, the carcass solutions were evaporated to 175 ml, or in a few cases to only 225 ml, and an aliquot of 25 ml was used for counting. The results were then adjusted to equivalent bases by the appropriate factor. Due to the high fat content of some of the carcasses, it was difficult to prevent separation of fat in the cooled solution. Minor variations in carcass counts may have resulted because of this, although inde-

pendent measurements showed that the fat contained negligible radioactivity.

All counts were corrected to a zero time base by the relation $A_t = A_o e^{-\lambda t}$ where A_t is the activity at time t, A_o is the activity at zero time base, and λ is the disintegration constant $\left(\frac{.693}{t_{\frac{1}{2}}}\right)$ where $t_{\frac{1}{2}}$ is the half life of Fe^{59}. Times were computed in hours from the zero time base (3 P.M., July 23, 1953) to the time the sample was counted. For example, for the liver of rat #16, A_t = 11314 cpm, t = 500 hours, $e^{-\lambda t}$ = .7359, and A_o is computed to be 15374 cpm.

Comparative exploratory studies, not reported herein, indicated that the counting technique used, considering the facilities and equipment available, gave results far superior to any of the dry methods of counting which were feasible.

Chemical. Routine hemoglobin determinations were performed by employing the method of Evelyn and Malloy (3) modified to use 0.02 ml of blood directly from the animal. Density of the cyanmethemoglobin was measured in a Coleman Model 14 Spectrophotometer at 540 millimicrons, using a set of matched 19 x 140 mm pyrex test tubes. Hemoglobin determinations were standardized by the method of Wong (23) as outlined by Hawk et al. (9). Standardization density measurements were on the Coleman Model 14 Spectrophotometer at 480 millimicrons. Normal blood for the standardization was obtained from rat #11 which had been on the basal diet for the 77 day duration of the experiment but was then placed on stock diet and held for a period of 80 days until blood values had returned to normal.

Hematocrit values were determined by the method of Van Allen (21),

by centrifuging for 15 minutes at 3000 rpm, in an International No. 2 Centrifuge.

Results

In Table 1, there is tabulated the average specific activity of tissues upon which these results are based. Tables A1 and A2 in the appendix give the total counts, tissue weights and specific activity of individual tissues upon which the average data is based.

1. The blood content of Fe^{59} was greater in every case in the iron deficient rats than in the corresponding iron supplemented rats, (Fig. 1).
2. The blood content of Fe^{59} was greater in every case in iron deficient rats receiving fluoride ion than in control rats receiving no added fluoride ion and in those control rats receiving added fluoride ion, (Fig. 1).
3. The blood content of Fe^{59} was greater in every case for rats receiving fluoride ion as a part of the diet or by stomach tube than for rats in corresponding groups receiving no added fluoride ion, (Fig. 1).
4. The blood content of Fe^{59} in iron supplemented rats was only slightly greater at 72 hours following doses of Fe^{59} at 0, 24, and 48 hours than the blood content of Fe^{59} in iron supplemented rats at 24 hours receiving an equal single dose at 0 hours, (Fig. 1).
5. The blood content of Fe^{59} in iron deficient rats in the absence

of added fluoride ion demonstrated a consistent increase with time through 48 hours following administration at which time the blood level of Fe^{59} apparently remained constant or suffered a slight decrease (Fig. 1).

6. The blood content of Fe^{59} in iron deficient rats receiving added fluoride ion increased with time through 48 hours following administration. At all time intervals through 72 hours following administration these values were greater than for corresponding rats receiving no added fluoride ion. At the end of 48 hours the blood content of Fe^{59} in rats receiving added fluoride ion also demonstrated a leveling-off or slight decrease.

7. With the possible exception of the liver and carcass specimens the Fe^{59} content of other tissues examined did not appear to differ greatly for iron supplemented as compared to iron deficient rats. The carcass and liver content of Fe^{59} for iron deficient rats receiving no added fluoride appeared to be greater than these values in the corresponding iron supplemented rats.

8. The Fe^{59} content of other tissues examined did not appear to differ greatly for all rats receiving added fluoride ion and those rats receiving no added fluoride ion.

9. The tissue uptake of Fe^{59} at 24 hours after administration was not greatly different between iron supplemented animals and iron deficient animals regardless whether fluoride ion was added or was not added. However, at 72 hours following administration the tissue uptake of Fe^{59} was greater in every case in the iron deficient rats than in the corresponding iron supplemented rats.

Here again the presence or absence of added fluoride ion did not appear to be a factor.

10. On the basis of these results and at the levels of Fe (III) ion, Fe^{59}(III) ion and fluoride ion fed and administered, the presence of fluoride ion in the diet or administered in the presence of Fe^{59}(III) ion did not produce anemia in the rat. On the contrary, it appeared that uptake of Fe^{59} by blood at least, in the rat, was enhanced.

11. Hematocrit and hemoglobin values (Tables 2 and 3) showed no significant differences for groups receiving sodium fluoride. These results substantiate those obtained by McClure and Kornberg (14) and are in opposition to those obtained by Ginn and Volker (4). The data also indicate that a reasonable degree of depletion was achieved in the animals on diets containing little or no iron.

12. Weights of all animals used in the experiment are recorded in Table 4. Reduced early rates of gain were noted in the animals receiving a sodium fluoride supplemented diet (Fig. 2). These data do not enter directly into the consideration of Fe^{59} uptake.

Table 1. Average specific activity of tissues.

Diet and Group	Hours between Fe^{59} administration and sacrifice	Specific activity (counts per minute per gram)				
		Carcass	Liver	Gut	Spleen	Blood
Iron deficient						
Group I	8	461	2031	1722	13373	3847
" III	24	610	5858	2463	14472	6775
" VII	48	984	5804	1968	13751	17773
" IX	72	841	4902	1651	7618	15073
Iron deficient administered fluoride						
Group II	8	681	4186	2070	24804	7330
" IV	24	489	4116	1225	8510	13845
" VIII	48	867	6698	1590	11281	20185
" X	72	978	5434	2176	7996	18784
Iron supplemented						
Group V	24	360	2153	2527	13095	2200
" XI	72	309	1617	939	2861	2653
Iron and fluoride supplemented						
Group VI	24	772	3356	2341	17453	5043
" XII	72	359	1975	1241	7747	6774

Note: Groups were fed diets and administered radioactive iron, and sodium fluoride solution, in accordance with schedules on pages 15,16, and 17.

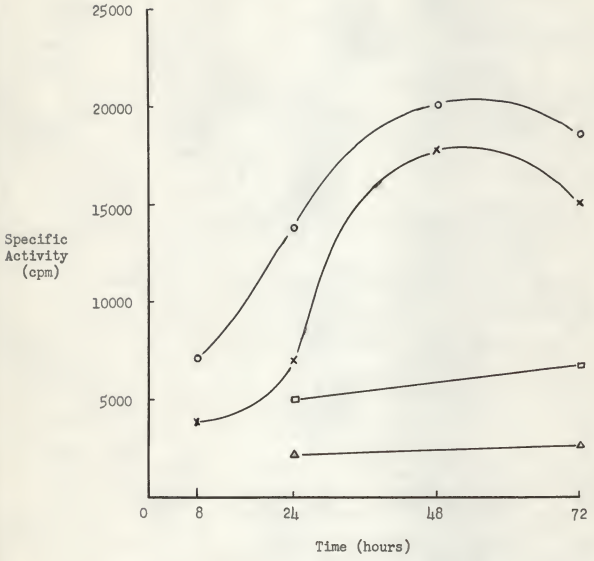


Fig. 1. Average specific activity of blood.

Table 2. Blood hematocrit and hemoglobin.

Group and diet	Rat No.	Hematocrit: Days on experiment diet 27	42	49	57	76	S*	Hemoglobin (gram/100 ml blood): Days on experiment diet 42	49	57	76	S*
Group I	18	—	—	48	51	44	—	—	13.1	12.6	11.1	--
Basal	19	—	—	40	33	35	22	—	14.4	7.8	8.2	8.2
	20	—	—	36	36	36	39	—	8.2	8.7	8.0	5.8
	21	—	—	33	37	38	—	—	8.0	8.0	8.2	—
Group II	22	45	39	--	38	35	42	7.3	--	8.0	7.8	7.5
Basal	23	—	28	—	37	35	31	9.3	—	8.2	7.3	6.7
	24	—	39	--	32	33	—	9.1	—	7.3	6.9	—
	50	—	—	—	39	40	—	—	—	10.0	8.0	—
Group III	26	—	—	--	36	35	31	--	—	7.8	7.8	8.4
Basal	27	—	46	—	33	33	—	10.4	—	8.7	6.7	6.2
	28	—	47	—	38	36	46	10.6	—	8.2	8.2	8.2
	29	—	36	--	35	34	44	8.4	—	7.1	7.3	7.1
Group IV	25	—	—	42	41	37	42	—	11.3	9.1	12.4	7.5
Basal	32	—	41	--	40	41	40	8.9	—	8.4	9.1	—
	33	—	33	—	32	37	32	8.0	—	8.2	7.5	—
	40	—	31	—	30	32	—	6.2	—	6.0	6.4	—
Group V	1	53	51	—	52	54	52	14.9	--	10.9	15.1	17.7
Basal+Fe	2	53	57	—	52	53	50	13.3	—	15.3	15.5	17.1
	52	—	59	—	55	50	57	14.0	—	14.2	15.7	16.2
	4	—	—	—	51	46	56	—	—	14.6	13.8	17.3
Group VI	9	47	--	—	55	51	51	—	—	12.9	14.9	16.9
Basal+Fe+F	10	54	--	—	54	52	51	—	—	14.2	12.6	13.3
	12	52	--	—	55	50	—	—	—	14.6	14.2	12.6
	13	48	--	—	52	49	—	—	—	17.5	13.8	—
Group VII	34	44	40	—	37	39	23	8.4	—	7.5	8.9	--
Basal	35	47	42	—	41	31	32	10.0	—	8.4	8.2	—
	36	54	41	—	36	35	39	11.5	—	9.3	8.2	—
	37	44	40	—	37	35	34	9.5	—	8.2	7.1	—
Group VIII	38	—	—	43	36	34	33	—	10.2	8.0	7.5	—
Basal	39	—	—	46	33	33	29	—	5.1	9.3	6.2	—
	41	—	—	46	43	36	28	—	12.0	—	7.1	—
	42	—	—	38	33	23	41	—	8.7	7.5	8.4	—
Group IX	43	—	—	42	40	42	—	—	12.9	9.3	12.6	11.8
Basal	51	—	—	39	35	37	35	—	10.6	7.5	8.2	8.9
	53	—	—	40	36	38	35	—	10.4	13.3	9.3	9.5
	3	—	—	38	37	38	16	—	10.9	7.8	9.8	9.1
Group X	46	—	—	39	39	37	—	—	10.2	8.4	8.0	8.0
Basal	47	—	—	43	42	39	44	—	11.8	9.5	10.4	10.6
	48	—	—	39	43	38	35	—	12.0	11.1	9.3	9.1
	49	—	—	41	44	27	32	—	10.4	17.7	8.9	8.4
Group XI	5	—	54	—	54	50	51	13.3	--	15.7	18.4	15.3
Basal+Fe	6	—	51	—	52	50	54	12.4	--	16.9	16.4	14.6
	7	—	51	—	49	47	55	11.8	—	12.2	12.9	16.6
	8	—	50	—	54	52	49	13.3	—	15.7	16.2	14.6
Group XII	14	—	53	—	54	47	48	13.1	—	14.6	12.9	12.2
Basal+Fe+F	15	—	53	—	55	54	54	13.8	—	16.2	16.9	21.1
	16	—	52	—	52	51	51	13.8	—	15.5	13.8	15.5
	17	—	54	—	52	45	46	14.9	—	13.8	13.3	13.5

* At time of sacrifice.

Table 3. Average blood hematocrit and hemoglobin values at 76 days.

Diet	Hematocrit	Hemoglobin (grams/100 ml blood)
Iron deficient (32 animals)	35.7	8.4
Iron supplemented (8 animals)	50.2	15.5
Iron supplemented plus sodium fluoride (8 animals)	50.0	14.1

Table 4. Animal weights (grams).

Group and diet	Rat No.	Days on experimental diet			
		0	44	56	76
Group I	18	104	138	185	217
Basal	19	106	192	254	275
	20	112	164	225	265
	21	130	184	248	275
Group II	22	118	224	257	278
Basal	23	108	170	203	222
	24	110	130	155	174
	50	95	146	185	204
Group III	26	104	182	247	268
Basal	27	115	140	206	232
	28	120	165	222	227
	29	100	165	223	248
Group IV	25	118	165	212	227
Basal	32	110	168	200	183
	33	117	218	244	275
	40	97	174	204	200
Group V	1	102	138	180	212
Basal+Fe	2	123	132	194	230
	52	93	135	194	237
	4	110	134	190	230
Group VI	9	118	147	193	233
Basal+Fe+F	10	112	127	160	210
	12	122	93	102	146
	13	92	145	162	210
Group VII	34	120	163	188	190
Basal	35	128	193	235	237
	36	115	177	235	252
	37	105	170	212	232
Group VIII	38	115	153	210	245
Basal	39	96	148	183	208
	41	107	142	169	197
	42	113	184	233	247
Group IX	43	113	150	203	205
Basal	51	102	185	220	232
	53	112	182	230	237
	3	130	183	228	245
Group X	46	105	170	210	235
Basal	47	108	107	152	175
	48	113	170	197	204
	49	120	175	220	254
Group XI	5	118	193	238	245
Basal+Fe	6	125	196	220	220
	7	112	173	198	228
	8	108	162	176	202
Group XII	14	113	154	203	258
Basal+Fe+F	15	100	103	148	197
	16	128	144	183	230
	17	122	127	170	208

Table 5. Average animal weights (on experimental diets).

Diet	Weight (grams)				Average weight gain (grams)		
	0 days	44 days	56 days	76 days	0-44 days	44-56 days	56-76 days
Iron deficient (32 animals)	111	168	212	230	57	44	18
Iron supplemented (8 animals)	111	157	198	226	46	41	28
Iron supplemented plus sodium fluoride (8 animals)	114	130	165	214	16	35	49

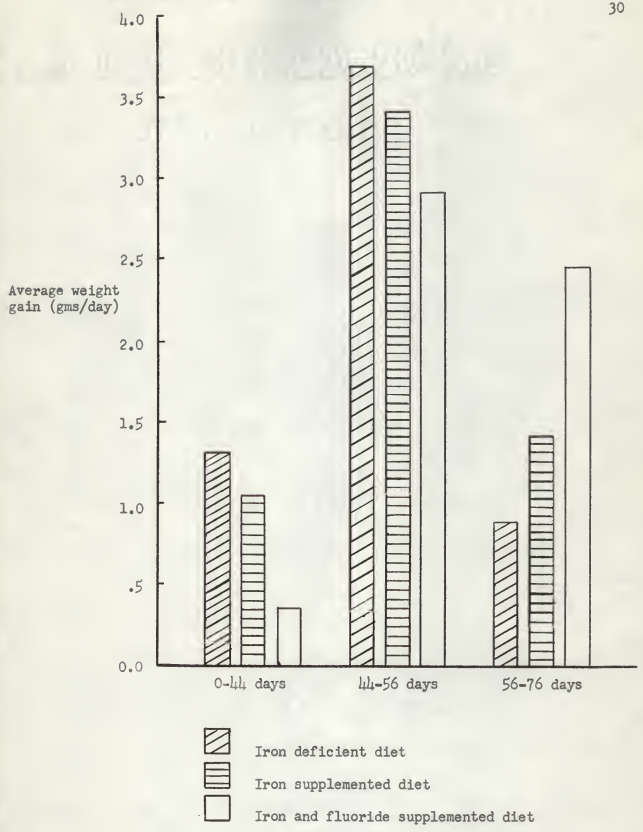


Fig. 2. Average weight gains on experimental diets.

DISCUSSION

Since all isotopes of iron are considered to be utilized in the same manner, it follows that absorption of non-radioactive iron should be identical with the pattern demonstrated by Fe^{59}.

The blood content of Fe^{59} was greater in iron deficient rats at every time interval investigated than it was in animals on the iron supplemented diet. This demonstrated a relative need for iron in the anemic rat. In addition, the iron supplemented rats demonstrated no increasing need for iron with time, on the basis of the Fe^{59} dosage administered. Within the 72 hour period studied the absorption of iron was not a function of time.

These data demonstrate that the presence of added fluoride ion, either directly in the stomach or in the diet, exert a significant effect upon the uptake of iron by blood.

The consistent increase in blood uptake of Fe^{59} by rats receiving fluoride ion, either by administration or in the diet, would indicate that the presence of fluoride ion enhances the absorption of iron. It is possible that fluoride ion may influence the iron absorption equilibria postulated by Hegsted et al. (11), may overcome the block to iron absorption postulated by Hahn et al. (7), or may enter into iron-fluoride complexes, because of the high formation constants reported in vitro by Dodgen and Rollefson (2) for these complexes, and be more readily absorbed in this form.

Within the limits of these experimental conditions the differences in Fe^{59} content of the other tissues investigated did not appear to be significant.

SUMMARY

Male rats on both iron supplemented and iron deficient diets were administered Fe^{59} to determine absorption of iron in the presence and in the absence of added fluoride ion.

Fluoride ion significantly enhanced blood uptake of Fe^{59}, when administered either by stomach tube or by inclusion in the diet, both in iron supplemented and iron deficient animals.

The blood uptake of Fe^{59} was not a function of time in iron supplemented animals but was definitely a function of time in iron deficient animals up to 48 hours at which time it leveled off or suffered a slight decrease.

The liver, gut, spleen, gut content, and residual carcass did not increase significantly in Fe^{59} content within 48 hours following administration of the Fe^{59} dose. However, comparisons of specific activity of tissues at the end of 72 hours indicated that tissue uptake of Fe^{59} is greater in the iron deficient rats.

The presence of fluoride ion in the diet at a level of 200 ppm does not produce anemia in the rat. When administered with Fe^{59} by stomach tube, fluoride ion does not produce anemia in the 72 hour period studied.

ACKNOWLEDGMENT

The author expresses grateful appreciation to Dr. J. S. Hughes, who initiated this project, to Dr. W. S. Ruliffson, for counsel and assistance during the course of the project, to Dr. R. E. Hein and Dr. R. H. McFarland for counsel and assistance in the radioactive phases of the study, and to the Moorman Manufacturing Company, Quincy, Illinois for financial support.

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APPENDIX

Table A1. Total activity counts and tissue weights.

Group	Rat #	Carcass		Liver		Gut		Spleen		Blood		Gut content, cpm	Excreta
		cpm	gms	cpm	gms	cpm	gms	cpm	gms	cpm	gms		cpm
I	18	52359	172	9155	7.91	14633	14.71	2820	.32	2344	1.02	45015	
	19	55572	207	17425	8.53	32381	15.65	9102	.67	9385	2.55	48223	27225
	20	132217	200	23142	8.00	36288	17.27	10102	.57	22772	4.09	107030	
	21	xx											
Average (3)		80049		16574		27767		7341		11500		66757	9075
II	22	142342	249	39279	10.37	33995	17.52	14923	.70	7510	1.35	49040	
	23	129526	182	35999	7.85	29761	13.52	18387	.65	10190	1.12	59088	
	24	xxx	118		5.17		10.77		.32		--		145955
	50	xxx	157		6.98		13.47		.60		.40		
Average (2)		135934		37639		31878		16605		8850		54089	36489
III	26	61121	197	52349	9.12	36209	14.00	11341	.56	4820	1.40	10613	
	27	146986	178	37935	6.63	28180	11.88	8267	.51	2435	.59	46469	25197
	28	162696	169	49139	7.22	36165	13.24	14413	.38	6122	.48	37403	
	29	49574	181	34745	6.73	30845	14.25	4513	.46	—	—	26437	
Average		105094		43542		32850		7134		3344		30230	6300
IV	25	95432	163	33160	6.64	237	11.73	4646	.43	6142	.37	71462	
	32	27960	131	11783	5.24	19244	10.70	2227	.38	3039	.31	128833	98588
	33	120246	217	36531	7.22	25651	13.80	4876	.55	12107	.80	95680	
Average (3)		81212		27258		15044		3916		7096		98658	32863

Table A1 (Cont'd.)

Group	Rat #	Carcass cpm	Carcass gms	Liver cpm	Liver gms	Gut cpm	Gut gms	Spleen cpm	Spleen gms	Blood cpm	Blood gms	Gut contents cpm	Excreta cpm
V	1	82154	165	25312	7.22	42171	11.43	4061	.30	1832	.55	99054	
	2	102870	209	18842	7.72	30379	12.91	7217	.31	1540	.68	140290	278740
	52	44554	190	13368	8.75	21882	13.33	5302	.57	4996	2.20	103995	
	4	31571	186	8713	7.67	27121	11.20	2755	.44	1138	1.22	53440	
Average		65287		16559		30388				2377		99195	69685
VI	9	72667	183	26413	6.78	19314	10.50	5045	.46	--	--	113154	
	10	153461	107	15882	5.39	28676	9.94	5088	.28	--	--	119491	81450
	12	78005	165	18933	5.87	24616	10.70	8127	.35	2370	.47	174204	
	13	xx	166		10.29		11.77		.55	--	--		
Average	(3)	101378		20409		24202		6087		2370		135616	27150
VII	34	170253	128	34896	5.13	27248	12.69	12546	.48	5716	.63	19386	
	35	145658	183	27594	5.68	21052	13.91	5221	.40	9696	.47	54190	19133
	36	149308	199	37718	7.25	28516	14.33	2541	.40	6248	.38	48981	
	37	137688	169	35458	5.58	28319	12.75	3690	.39	22204	.89	35117	
Average		150726		33917		26284		6000		10966		39419	4783
VIII	38	96862	181	34109	6.10	16538	12.90	3975	.52	10333	.60	66517	
	39	141816	148	42483	5.39	12050	11.14	2653	.45	--	--	66855	174178
	41	144416	135	33227	4.61	23864	11.02	5250	.35	5653	.21	85003	
	42	129014	185	39272	6.43	21705	11.90	6302	.38	21993	1.34	76848	
Average		128027		37273		18551		4545		12660		73806	43545

Table A1 (Concl.)

Group	Rat #	Carcass		Liver		Gut		Spleen		Blood		Gut Content, cpm	Excreta cpm
		cpm	gms	cpm	gms	cpm	gms	cpm	gms	cpm	gms		
IX	43	97685	141	29750	5.17	20441	10.78	3139	.33	14410	.88	20020	
	51*	90684	167	23953	5.51	16682	11.41	3934	.36	13045	.95	39009	239625
	53	121983	166	16592	4.98	21945	11.49	1746	.33	10563	3.94	53148	
	3*	127904	161	32294	5.23	15549	11.64	1423	.30	35207	1.77	38637	
Average		109564		25647		18654		2560		25806		37704	59906
X	46	71744	154	20557	4.68	18524	10.45	2411	.36	58658	4.07	65201	
	47	71477	108	21674	3.34	23187	8.69	1893	.19	17410	.81	41627	16554
	48	141179	147	27634	4.95	21184	9.99	2182	.31	33900	1.69	29530	
	49	160775	179	29719	5.64	22907	10.69	3232	.39	11886	.62	58781	
Average		111294		24896		21450		2430		30464		48785	4139
XI	5	38211	211	20320	11.12	17069	17.68	2661	.50	3731	1.73	93450	
	6	20082	171	9850	10.51	11723	17.49	685	.54	838	.48	85087	284297
	7	61154	177	18321	7.95	16885	10.80	1464	.41	1507	.32	89661	
	8	92476	167	9761	6.98	5890	10.52	462	.36	3180	1.59	434441	
Average		52981		14563		12892		1318		2314		77910	71074
XII	14	94512	195	20783	8.34	14962	12.87	195	.51	4724	.48	68097	
	15	51295	143	3373	5.77	13353	10.65	4594	.30	1977	.41	122723	36452
	16	52439	190	15374	6.14	14333	11.33	3732	.43	8510	1.25	102268	
	17	34784	159	14516	6.26	14862	11.59	2777	.42	3206	.57	117679	
Average		58258		13512		14378		2825		4604		102691	9113

* Probable slight loss of Fe^{59} dose.

Table A2. Specific activity of tissues.

Group, Diet and Treatment	Rat #	Specific activity (counts per min. per gram) Carcass	Liver	Gut	Spleen	Blood
Group I	18	316	1157	995	8812	2298
Basal ration	19	310	2043	2069	13585	3680
3½ μc Fe	20	759	2893	2101	17723	5568
Sacrificed at 8 hrs.						
Average (3)		461	2031	1722	13373	3847
Group II	22	599	3787	1940	21319	5563
Basal ration	23	763	4586	2201	28288	9098
3½ μc Fe+F sol.	24	xxx				
Sacrificed at 8 hrs.	50	xxx				
Average (2)		681	4186	2070	24804	7330
Group III	26	332	5740	2586	20252	3443
Basal ration	27	837	5722	2372	16210	4127
3½ μc Fe	28	996	6806	2731	11613	12754
Sacrificed at 24 hrs.	29	274	5163	2165	9811	--
Average		610	5858	2463	14472	6775
Group IV	25	622	5039	20	10805	16600
Basal ration	32	236	2249	1798	5860	9803
3½ μc Fe+F sol.	33	608	5060	1859	8865	15134
Sacrificed at 24 hrs.						
Average (3)		489	4116	1225	8510	13845
Group V	1	507	3506	3690	13537	3331
Basal ration + Fe	2	498	2441	2353	23281	2265
3½ μc Fe	52	258	1528	1642	9302	2271
Sacrificed at 24 hrs.	4	175	1136	2422	6261	933
Average		360	2153	2527	13095	2200
Group VI	9	397	3896	1839	10967	--
Basal ration +Fe+F	10	1434	2947	2885	18171	--
3½ μc Fe	12	486	3225	2300	23220	5043
Sacrificed at 24 hrs.	13	xxx				
Average		772	3356	2341	17453	5043

Table A2. (Concl.)

Group, Diet and Treatment	Rat #	Specific activity (counts per min. per gram) Carcass	Liver	Gut	Spleen	Blood
Group VII	34	1368	6802	2147	26138	9073
Basal ration	35	847	4858	1513	13052	20630
17 μc Fe at 0,24 hrs.	36	780	5202	1990	6352	16442
Sacrificed at 48 hrs.	37	941	6354	2221	9462	24948
Average		984	5804	1968	13751	17773
Group VIII	38	590	5592	1282	7644	17222
Basal ration	39	958	7882	1082	5896	—
17 μc Fe+F sol.	41	1110	7208	2166	15000	26919
Sacrificed at 48 hrs.	42	810	6108	1828	16584	16413
Average		867	6698	1590	11281	20185
Group IX	43	790	5754	1896	9512	16375
Basal ration	51	618	4347	1462	10928	13732
11.3 μc Fe at 0,24,	53	956	3332	1910	5291	10295
48 hrs.	3	1002	6175	1336	4743	19891
Sacrificed at 72 hrs. Average		841	4902	1651	7618	15073
Group X	46	824	4393	1773	6697	14412
Basal ration	47	817	6489	2668	9963	21494
11.3 μc Fe+F sol. ,	48	1177	5583	2121	7039	20059
at 0 ,24, 48 hrs.	49	1092	5269	2143	8287	19171
Sacrificed at 72 hrs. Average		978	5434	2176	7996	18784
Group XI	5	197	1827	965	5322	2157
Basal ration +Fe	6	121	937	670	1269	1746
11.3 μc Fe at 0,24,	7	350	2305	1563	3571	4709
48 hrs.	8	567	1398	560	1283	2000
Sacrificed at 72 hrs. Average		309	1617	939	2861	2653
Group XII	14	507	2492	1163	382	9842
Basal ration +Fe+F	15	371	585	1254	15313	4822
11.3 μc Fe at 0, 24,	16	319	2504	1265	8679	6808
48 hrs.	17	238	2319	1282	6612	5625
Sacrificed at 72 hrs. Average		359	1975	1241	7747	6774

THE INTERRELATION OF FERRIC IONS AND FLUORIDE IONS IN ANIMAL NUTRITION

by

LOREN VIRGIL BURNS

B. S., Washburn University, 1932

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1954

This study, prompted by discrepancies appearing in the literature, was an exploratory investigation designed to determine the influence of added fluoride ion upon the absorption and utilization of iron by the rat.

Weanling male rats were partially depleted of iron by feeding a diet of dried skimmed milk. Control groups were fed this diet supplemented with ferric chloride and with ferric chloride plus sodium fluoride. Hemoglobin and hematocrit values of the blood of all rats were determined at periodic intervals throughout the period of depletion, and at the time of sacrifice after the feeding of Fe^{59}.

All rats were administered a total of 34 microcuries of Fe^{59} by stomach tube. This amount of Fe^{59} was given in one dose for animals to be sacrificed at 8 and 24 hours, in two equal doses for animals to be sacrificed at 48 hours, and in three equal doses for animals to be sacrificed at 72 hours. Corresponding groups were also administered sodium fluoride solution by stomach tube immediately after the Fe^{59} dose. The rats were sacrificed and the liver, spleen, gut, and a small sample of blood were removed and weighed. Gut contents were washed out and collected separately but were not weighed due to the variable amounts of wash water used. All other body components were combined and treated as residual carcass. Each of the above tissues, and the carcass, were digested individually in nitric acid and adjusted to a specific volume for counting.

Radioactivity of each tissue and carcass was determined by a dipping counter tube. Counts were corrected for background, decay, and day-to-day variability of the counting assembly.

Rats receiving sodium fluoride, either by stomach tube or by admini-

stration in the diet, showed a consistently greater uptake of Fe^{59} by the blood when compared to corresponding rats not receiving the added sodium fluoride. It was concluded that fluoride ion significantly enhanced the blood uptake of iron both in iron deficient and iron supplemented rats.

The blood uptake of Fe^{59} was not a function of time in iron supplemented rats. The blood content of Fe^{59} of iron deficient rats receiving no added fluoride ion showed a consistent increase in Fe^{59} uptake through 48 hours after which the amount of Fe^{59} appeared to remain constant or to decrease slightly. In iron deficient rats which received added fluoride ion the blood content of Fe^{59} was greater than that of rats which did not receive added sodium fluoride but showed the same general relationships with time, rising to a peak around 48 hours then decreasing slightly by 72 hours. The Fe^{59} content of other tissues examined did not appear to be related to the presence or absence of added fluoride ion.

Further, the results of this experiment indicate that the ingestion by rats of fluoride ion as sodium fluoride does not result in anemia.